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Respiratory Viral Shedding in Healthcare Workers Reinfected with SARS-CoV-2, Brazil, 2020

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DOI: https://doi.org/10.3201/eid2706.210558

We documented 4 cases of severe acute respiratory syndrome coronavirus 2 reinfection by non–variant of concern strains among healthcare workers in Campinas, Brazil. We isolated infectious particles from nasopharyngeal secretions during both infection episodes. Improved and continued protection measures are necessary to mitigate the risk for reinfection among healthcare workers.

Coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China,

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in late 2019. As of April 8, 2021, COVID-19 has affected >132 million persons and caused >2.87 million deaths around the world (https://covid19.who.int). Whether the immune response elicited by an initial infection protects against reinfection is uncertain. The Pan American Health Organization provisionally defines reinfection as a positive SARS-CoV-2 test result ≥45 days after initial infection, given that other infections and prolonged shedding of SARS-CoV-2 or viral RNA have been ruled out (*1*). Healthcare workers (HCWs) are consistently exposed to SARS-CoV-2 and are therefore susceptible to reinfection.

We investigated 4 cases of SARS-CoV-2 reinfection among HCWs at the Hospital das Clínicas da Unicamp, a tertiary public hospital at the University of Campinas (Campinas, Brazil). This study was approved by the Research Ethical Committee of the University of Campinas (approval no. CAAE-31170720.3.0000.5404). The 4 HCWs, consisting of 3 nurses and 1 staff member, were women with an average age of 44 years (range 40–61 years) (Figure 1, panel A). For the initial infections, symptom onset ranged from April 5–May 10, 2020, and lasted 10–23 days. We identified SARS-CoV-2 RNA in nasopharyngeal swab samples using real-time quantitative reverse transcription PCR (qRT-PCR) 2–4 days after symptom onset (*2*). All 4 HCWs had mild COVID-19 signs and symptoms and recovered (Table). After signs and symptoms resolved, the HCWs tested negative by qRT-PCR, Elecsys Anti-SARS-CoV-2 (Roche Diagnostics, https://diagnostics.roche.com), or both. Reinfection, confirmed by a nucleic acid amplification test using the GeneFinder COVID-19 Plus RealAmp Kit (*3*), developed 55–170 days after symptom onset of the first infection. Signs and symptoms of reinfection lasted 9–23 days. Only 1 HCW had a concurrent condition (chronic bronchitis), and none were immunosuppressed. None required hospitalization during the initial or reinfection episodes (Table). After recovering from their initial infections, all HWCs continued to use the same types of personal protective equipment (i.e., disposable surgical masks, gloves, gowns, and goggles) in accordance with recommendations from the Ministry of Health of Brazil (https://coronavirus.saude.gov.br/saude-e-seguranca-do-trabalhador-epi).

To assess whether infectious SARS-CoV-2 particles were shed in nasopharyngeal secretions during both COVID-19 episodes, we conducted viral isolation in Vero cells (ATCC no. CCL-81) (W.M. de Souza, unpub. data, http://dx.doi.org/10.2139/ssrn.3793486) (Appendix). We inoculated Vero cells with isolated SARS-CoV-2 virions from nasopharyngeal swab samples collected during the first and second infections; we observed a cytopathic effect 3–4 days after inoculation. On day 4, we obtained cell culture supernatant by centrifugation and conducted qRT-PCR selective for the envelope gene to confirm the presence of SARS-CoV-2 RNA; we found the supernatants had 2.8×10^2–1.4×10^{10} RNA copies/mL (*2*).

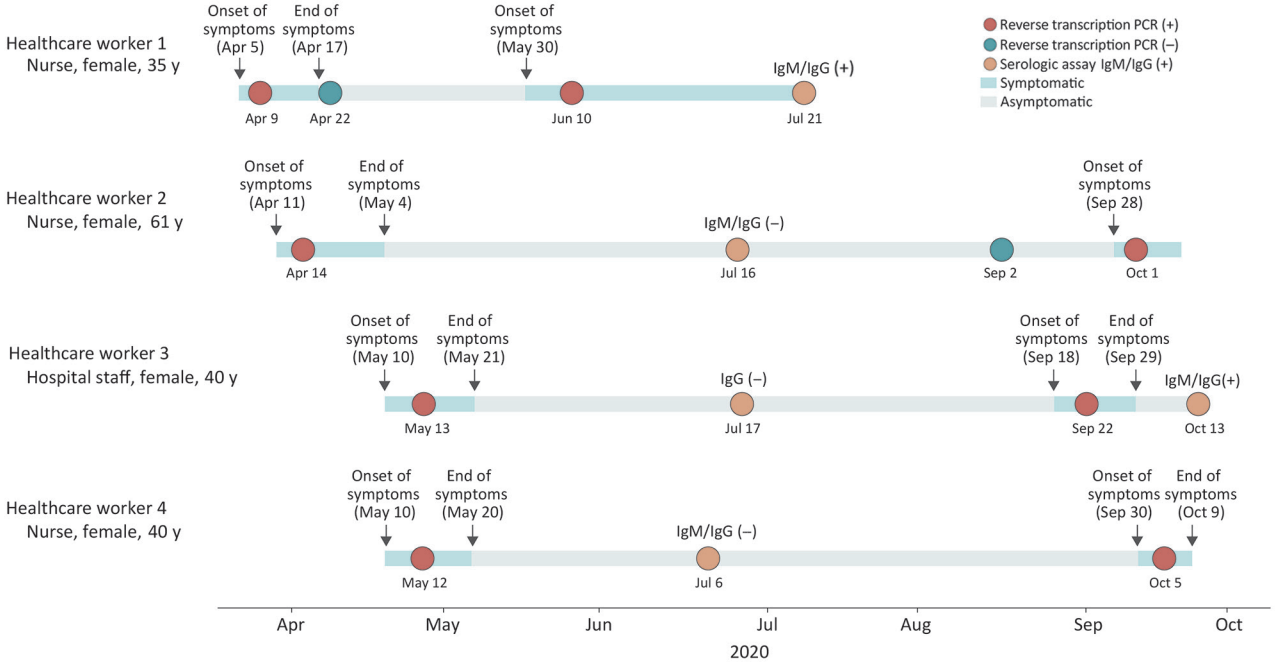

Figure. Timeline of severe acute respiratory syndrome coronavirus 2 reinfections (SARS-CoV-2) among healthcare workers, Brazil, 2020. (+), positive; (–), negative.

Table. Characteristics of healthcare workers with severe acute respiratory syndrome coronavirus 2 reinfections, Brazil, 2020*

Characteristic	Healthcare worker			
	1	2	3	4
Underlying conditions	None	Chronic bronchitis	None	None
Hospitalized	No	No	No	No
Symptoms				
First infection	Fever, headache, chills, sneezing, coryza, and myalgia	Headache, cough, myalgia, odynophagy, coryza, diarrhea, and ageusia	Nasal congestion, coryza, cough, ageusia	Fever, headache, myalgia, coryza, dry cough, vomiting, and malaise
Second infection	Headache, nasal congestion, odynophagia, ageusia, and anosmia	Cough, myalgia, odynophagia, anosmia, and diarrhea	Odynophagia, sneezing, coryza, diarrhea, ageusia, and anosmia	Odynophagia, dry cough, myalgia, malaise, coryza, and headache
Cycle threshold values				
First infection†	E gene: 35.24; N gene: 40.12	E gene: 31.8	E gene: 35.15	E gene: 34.80; RdRp gene: 39.86
Second infection‡	E gene: 31.14; N gene: 31.3; RdRp gene: 32.58	E gene: 20.45; N gene: 20.52; RdRp gene: 22.65	E gene: 26.04; N gene: 26.88; RdRp gene: 28.40	E gene: 23.72; N gene: 23.48; RdRp gene: 25.67
Time between symptom onsets, d	55	170	131	148

*E gene, envelope gene; N gene, nucleoprotein gene; RdRp gene, RNA dependent RNA polymerase gene.
†Real-time quantitative reverse transcription PCR selective for the envelope gene (*2*).
‡Nucleic acid amplification test using the GeneFinder COVID-19 Plus RealAmp Kit (OSANG Healthcare Co. Ltd., http://www.osanghc.com) (*3*).

We confirmed viral isolation by the increased number of RNA copies per milliliter and the decreased cycle threshold values after passage into Vero cells. The isolation of SARS-CoV-2 shows that nasopharyngeal swab samples contained infectious particles during both COVID-19 episodes.

SARS-CoV-2 variants of concern (VOCs; i.e., lineages B.1.1.7, B.1.351, and P.1.), and particularly their mutations in the spike protein, have been associated with reinfection (*4*,*5*). To investigate this association, we sequenced SARS-CoV-2 genomes from samples or isolates in this study using the ARTIC version 3 protocol (https://artic.network/ncov-2019) with MinION sequencing (Oxford Nanopore Technologies, https://nanoporetech.com). We obtained sequences with 66%–99% genome coverage (mean depth >20-fold) for 3 of 4 HCWs (Appendix Figure, https://wwwnc.cdc.gov/EID/article/27/6/21-0558-App1.pdf). We submitted the sequences to GISAID (https://www.gisaid.org; accession nos. EPI_ISL_1511399, EPI_ISL_1511603, EPI_ISL_1511641, and EPI_ISL_1511644). We used the Pangolin COVID-19 Lineage Assigner tool (*6*) to classify samples as members of lineages B.1.1.28 (n = 3) and B.1.1.33 (n = 1); 3 of these samples were taken during the reinfection episodes of HCWs 1, 2, and 4 and 1 during the first episode of HCW 1 (Appendix Figure). These lineages have circulated in Brazil since early March 2020 (*7*) and have not been associated with reinfection or long-term infection. In addition, we found the D614G mutation in the spike protein in samples from both episodes of HCW 1 and the second episode of HCW 2. The D614G mutation has been associated with enhanced viral replication in the upper respiratory tract and increased susceptibility of the virus to neutralization by antibodies (*8*). In addition, we found the V1176F mutation in the spike protein in samples from both episodes of HCW 1 and the second episode of HCW 4; however, the effects of this mutation remain unclear. None of the genomes had the mutations in spike proteins described in 3 recent VOCs (https://cov-lineages.org). Other cases of SARS-CoV-2 reinfection by strains without mutations in the spike protein were documented in India; those infections were associated with lineages B.1.1.8 and B.1.1.29 (*9*). Our results provide additional evidence of SARS-CoV-2 reinfection by non-VOC strains.

In conclusion, we report cases of SARS-CoV-2 reinfection among HCWs. We observed the shedding of infectious viral particles during both infection episodes of each HCW. Hence, the continuation of protective measures, as well as efforts to monitor, track exposures, and identify areas at high risk for infection, are critical to reducing SARS-CoV-2 reinfection, especially among HCWs.

Acknowledgments

We thank Thermo Fisher Scientific, which kindly provided an EVOS inverted microscope for the Biosafety Level 3 facility at Laboratório de Estudos de Vírus Emergentes. We also thank the UNICAMP Task Force Against COVID-19, which facilitated this study. We thank Ministry of Science, Technology and Innovation of Brazil (MCTI) and all members of the Corona-ômica network for financial support. We thank Lucy Matkin for proofreading the text.

This study was supported by grants from São Paulo Research Foundation (FAPESP; grant nos. 2016/00194-8 and 2020/04558-0) and Fundo de apoio ao ensino, pesquisa e extensão da UNICAMP (grant no. 2266/20). This study was also supported by MCTI through the Rede Corona-ômica Brazil/MCTI (funded by the Financier of Studies and Projects [FINEP] grant no. 01.20.0003.00), RedeVírus/MCTI (FINEP grant no. 01.20.0029.000462/20), and the Brazilian National Council for Scientific and Technological Development, CNPq, grant no. 404096/2020-4). This project was supported by the Medical Research Council and FAPESP–Brazil–UK Centre for (Arbo)virus Discovery, Diagnosis, Genomics and Epidemiology partnership award (grant nos. MR/S0195/1 and FAPESP 2018/14389-0). W.M.S. is supported by FAPESP (grant nos. 2017/13981-0 and 2019/24251-9) and CNPq (grant no. 408338/2018-0). N.R.F. is supported by a Wellcome Trust and Royal Society Sir Henry Dale Fellowship (grant no. 204311/Z/16/Z). K.B.S., CLS., and P.L.P were supported by FAPESP fellowships (grant nos. 2020/02159-0, 2020/02448-2, and 2017/26908-0). M.R.A. was supported by Coordination for the Improvement of Higher Education Personnel fellowships. D.A.T.T. and L.S.M. were supported by CNPq fellowships (grant nos. 141844/2019-1 and 382206/2020-7).

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Multisystem Inflammatory Syndrome in Adults after Mild SARS-CoV-2 Infection, Japan

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DOI: https://doi.org/10.3201/eid2706.210728

In Japan, a 51-year-old man had minimally symptomatic severe acute respiratory syndrome coronavirus 2 infection. Multisystem inflammatory syndrome was diagnosed ≈5 weeks later; characteristics included severe inflammation, cardiac dysfunction, and IgG positivity. Clinicians should obtain detailed history and examine IgG levels for cases of inflammatory disease with unexplained cardiac decompensation.

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Article DOI: https://doi.org/10.3201/eid2706.210558

Respiratory Viral Shedding in Healthcare Workers Reinfected with SARS-CoV-2, Brazil, 2020

Appendix

Materials and Methods

Sample Collection and Ethics

We used residual serum and nasopharyngeal swab samples collected at distinct time points corresponding to the period when patients had coronavirus disease (COVID-19) symptoms. Data, including age, sex, occupation, sample collection data, symptoms, and concurrent conditions, were collected from electronic health records (Appendix Table).

SARS-CoV-2 Diagnosis

Viral RNA was extracted using the Quick-RNA viral kit (Zymo Research, https://www.zymoresearch.com) following the manufacturer recommended procedures. The RNA from samples were tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time quantitative reverse transcription PCR selective for the envelope gene and nucleic acid amplification test using the GeneFinder COVID-19 Plus RealAmp Kit (OSANG Healthcare Co. Ltd., http://www.osanghc.com) (Appendix Table) (*2*,*3*). In addition, the IgM and IgG antibodies against SARS-CoV-2 proteins were measured by Elecsys Anti-SARS-CoV-2 (Roche Diagnostics, https://diagnostics.roche.com), according to the manufacturer instructions.

Virus Isolation

Nasopharyngeal lavage samples were inoculated into Vero cells (CCL-81) for virus isolation using the method described previously (W.M. de Souza, upub. data, https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3793486). Briefly, 5×10^5 cells/mL of Vero cells were plated in a T225 flask with Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 1% of 10,000 units of penicillin and 10 mg of streptomycin/mL solution (Sigma-Aldrich Inc., https://www.sigmaaldrich.com). Next, samples were thawed on ice, diluted 1:10 in DMEM, and centrifuged at 12.000 × g for 5 min

at 4°C. Samples were filtered using 0.22 μm syringe filters, and incubated on ice for 1 h with a solution of DMEM with 10,000 units of penicillin, 10 mg of streptomycin/mL, and 250 μg/mL amphotericin B (i.e., 1:1 ratio) (Sigma-Aldrich, Inc.) in a final dilution of 1:10. After incubation at 37°C for 1 h, the inoculum was removed and replaced with fresh culture medium. Cells were incubated at 37°C and observed for cytopathic effects daily up to 4 days. Supernatant was collected daily and viral replication was confirmed through the increase of cycle threshold value using real-time quantitative reverse transcription PCR (*2*). All experiments related to culture cells and viral replication were performed in the Biosafety Level 3 laboratory at the Emerging Viruses Laboratory of the University of Campinas, Campinas, Brazil.

SARS-CoV-2 Genome Sequencing and Analysis

SARS-CoV-2 genome sequencing was carried out using the ARTIC version 3 (https://artic.network/ncov-2019) protocol with MinION sequencing (Oxford Nanopore Technologies, https://nanoporetech.com). cDNA was synthesized using the extracted RNA with random hexamers and the Protoscript II First Strand cDNA synthesis Kit (New England Biolabs, https://www.neb.com). Then, we performed the SARS-CoV-2 whole-genome multiplex-PCR amplification using ARTIC network SARS-CoV-2 V3 primer scheme and Q5 High-Fidelity DNA polymerase (New England Biolabs). The PCR product was purified using AMPure XP magnetic beads (Beckman Coulter, Inc., https://www.beckmancoulter.com), according to manufacturer instructions. DNA was quantified using Qubit dsDNA High Sensitivity assay on the Qubit 3.0 (Thermo Fischer Scientific, https://www.thermofisher.com). To uniform sequencing, equimolar normalization of 10 ng per sample was performed followed by barcoding using the EXP-NBD104 Native Barcoding Kits (Oxford Nanopore Technologies). Then, barcoded samples were pooled followed by library preparation using the SQK-LSK109 Kit (Oxford Nanopore Technologies). Finally, Nanopore sequencing libraries were loaded onto an R9.4.1 flow-cell (Oxford Nanopore Technologies) and sequenced using MinKNOW version 20.10.3 (Oxford Nanopore Technologies). FAST5 files containing the raw signal data were basecalled, demultiplexed, and trimmed using Guppy version 4.4.1 (Oxford Nanopore Technologies). The reads were aligned against the reference genome Wuhan-Hu-1 (GenBank accession no. MN908947) using minimap2 version 2.17.r941 (https://github.com/lh3/minimap2) and converted to a sorted BAM file using SAMtools (http://samtools.sourceforge.net). Length filtering, quality test, primmer trimming, variant calling and consensus sequences were performed for each

barcode using guppyplex from ARTIC (https://artic.network/ncov-2019). Genome regions with a depth of <20-fold were represented with N characters. Tablet alignment viewer (version 1.19.09.03) (https://ics.hutton.ac.uk/tablet) was used to visualize the mapped sequence. Finally, the 2 genomes were uploaded to the CoV-GLUE online resource (J. Singer, unpub. data, https://www.preprints.org/manuscript/202006.0225/v1) for mutation determination. The sequences have been uploaded to GISAID under the accession nos. EPI_ISL_1511399, EPI_ISL_1511603, EPI_ISL_1511641, and EPI_ISL_1511644.

HCW 1 (first infection)
EPI_ISL_1511641 (coverage = 99.4%)

Gene	Nucleotide	Amino acid
nsp7	C12053T	L71F
nsp12	C14408T	P323L
nsp15	C20132T	A171V
S	A23403G	D614G
	G25088T	V1176F
N	G28881A,G28882A	R203K
	G28883C	G204R

HCW 1 (second infection)
EPI_ISL_1511644 (coverage=97.0%)

Gene	Nucleotide	Amino acid
nsp7	C12053T	L71F
nsp12	C14408T	P323L
nsp15	C20132T	A171V
S	A23403G	D614G
	G25088T	V1176F
N	G28881A,G28882A	R203K
	G28883C	G204R

HCW 2 (second infection)
EPI_ISL_1511399 (coverage = 97.2%)

Gene	Nucleotide	Amino acid
nsp2	C1601T	L266F
	G2447T	G548C
nsp6	G11317T	M115I
nsp12	C14408T	P323L
nsp14	G18255T	M72I
S	A23403G	D614G
	G23587C	Q675H
E	C26447T	S68F
M	G26763T	A81S
ORF 6	T27299C	I33T
N	G28881A, G28882A	R203K
	G28883C	G204R
	T29148C	I292T
	G29513T	A414S

HCW 4 (second infection)
EPI_ISL_1511603 (coverage = 66.3%)

Gene	Nucleotide	Amino acid
nsp2	A1682C	I293L
nsp6	C11036T	L22F
nsp12	C14408T	P323L
S	G25088T	V1176F
N	G28655C	D128H
	G28881A,G28882A	R203K
	G28883C	G204R

Appendix Figure. Severe acute respiratory syndrome coronavirus mutations in 3 of 4 reinfected HCWs, Brazil, 2020. Wuhan-Hu-1 (GenBank accession no. MN908947) used as reference sequence. E, envelope protein; HCW, healthcare worker; M, matrix protein; N, nucleoprotein; S, spike protein.